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Approval Signatures

Kathryn M. Reynolds	4/16/08
Kathryn Reynolds, Author	Date
Phil Hansen	4/16/2008
Phillip Hansen, Technical Program Manager	Date
Louise R. Ogden	16 April 2008
Louise Ogden, Quality Assurance Officer	Date

1. Introduction

1.1 Scope

This is a method for the extraction and analysis of certain pesticides in drinking well water using the Horizon SPE-DEX® 4790 automated extraction system and analysis by GC/MS with the APEX ProSep large volume injector for the USDA Pesticide Data Program.

1.1.1 The following compounds have been validated or under study by this method.

Compound	CAS Number	Compound	CAS Number
Acetochlor	34256-82-1	Metolachlor	51218-45-2
Alachlor	15972-60-8	Metribuzin	21087-64-9
Atrazine	1912-24-9	Myclobutanil	88671-89-0
Boscalid	188425-85-6	Pendimethalin	40318-45-4
Chlorothalonil[1]	1897-45-6	Phorate	298-02-2
Chlorpyrifos	2921-88-2	Phorate oxon	2600-69-3
Cis-permethrin	52341-33-0	Phorate sulfone	2588-04-7
Clomazone	81777-89-1	Phorate sulfoxide	2588-03-6
Cyanazine	21725-46-2	Prometon	1610-18-0
DCPA (Dacthal)	1861-32-1	Propachlor	1918-16-7
Deethylatrazine	6190-65-4	Propanil	709-98-8
Deisopropylatrazine	----------	Propazine	139-40-2
Diazinon	333-41-5	Propiconazole	60207-90-1
Dichlobenil	1194-65-6	Simazine	122-34-9
Dimethenamid	87674-68-8	Tebuconazole	107534-96-3
Dimethoate	60-51-5	Tebuprimiphos	96182-53-5
EPTC	759-94-4	Tebuthiuron	34014-18-1
Ethalfluralin	55283-68-6	Terbufos	13071-79-9
Fluometuron	2164-17-2	Tetraconazole	112281-77-3
Fonofos	944-22-9	Trans-permethrin	52341-32-9
Malathion	121-75-5	Triallate	2303-17-5
Malathion oxon	1634-78-2	Trifluralin	1582-09-8
Methyl Parathion	298-00-0	Triticonazole	131983-72-7
Methidathion	950-37-8		

Chlorothalonil spikes show significant loss within 7 days under sample storage conditions outlined in this method.

1.1.2 The LOD (limit of detection) and LOQ (limit of quantitation) for each compound are listed in Table 1.

1.2 Summary

A 1 liter bottled water or ground water sample is poured into a 1L amber bottle and placed on the Horizon SPE-DEX® 4790. The appropriate extraction method is loaded into the Horizon controller that will automatically condition a hydrophyllic speedisk with acetone, ethyl acetate, hexane, methanol and water. The sample is pulled through the speedisk, and the speedisk allowed to air dry. Pesticides are automatically eluted with small rinses of acetone, ethyl acetate and hexane. The extract is vacuum filtered through a Horizon Dry Disk membrane to remove the excess water and then concentrated by evaporation using a turbo-vap. An internal standard mixture is added and the extract is brought to final volume with hexane. The final extract is analyzed by gas chromatography / mass spectroscopy using an APEX ProSep injector. Analytes are identified by comparing their measured mass spectra and retention times to reference spectra and retention times from calibration standards. The concentration of each analyte is measured by relating the MS response of the analyte quantitation ion with the quantitation ion of the internal standard.

1.3 Definitions

1.3.1 Horizon SPE-DEX® 4790 Automated Extractor allows for the partition of pesticides from a water sample onto a sorbent material (hydrophilic divinyl benzene speedisk, DVB). The pesticides are eluted from the solid sorbent with organic solvents. This process is fully automated using a vacuum pump system and nitrogen gas to control pneumatics.

1.3.2 APEX ProSep™ is an injector that allows for large volume injections and is designed to separate target analytes from the solvent before the bands reach the bottom of the pre-column. The pneumatic control allows for the optimization of flow, temperature and pressure profiles independent of the GC while protecting the GC's pneumatics from large volumes of solvent.

1.3.3 Process Control: Analyte (Metazachlor) that is unlikely to be found in any sample. A known quantity is added to every sample before processing and is measured by the same procedure as all other analytes. The purpose of the process control is to monitor method performance of each sample.

1.3.4 Internal Standard: Known quantity of pure analyte(s) added to final extract and used to measure relative responses of other analytes in the same solution.

1.3.5 Reagent Blank: 1 L reagent water that is treated exactly as samples including addition of surrogate, exposure to all glassware, equipment, solvents and internal standards. The purpose of the blank is to determine if any analytes or interferences are present.

1.3.6 Matrix Blank: 1 L bottled water (Evian® brand) or ground water is treated exactly as sample including addition of surrogate, exposure to all glassware, equipment, solvents and internal standards. The purpose of the blank is to determine if any analytes or interferences are present in the bottled water or ground water matrix.

1.3.7 Matrix Spike: 1 L bottled water (Evian® brand) or ground water to which known quantities of specified analytes are added in the laboratory prior to processing and treated exactly like all samples. The purpose of the spike is to determine if the method is in control.

1.3.8 Stock Standard: A concentrated 1 mg mL^{-1} solution containing one analyte prepared in the laboratory using neat standards supplied by the EPA, manufacturing companies or purchased from a reputable commercial source.

1.3.9 Primary Dilution Standard Solution (PDS): A 10 ppm solution of numerous analytes prepared from the individual stock standards and diluted to prepare calibration standards.

1.3.10 Calibration Standards: A solution prepared from the primary dilution standard solution, internal standards and surrogate. Calibration standards are used to make the calibration curves for each analyte at various concentration levels.

1.4 References and Attachments

1.4.1 Minnesota Department of Agriculture. Method EAW101

1.4.2 EPA Method 525.2. Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography / Mass Spectrometry.

1.4.3 Horizon Technology. SPE-DEX® 4790 Series Extractor: Automated Solid Phase Extractor System User's Guide.

1.4.4 Attachments

- Table 1: Limit of Detection (LOD) and Limit of Quantitation (LOQ) for PDP analytes is listed in ppt.
- Table 2: Retention Time and Mass Analysis.
- Attachment I: Operation of the Horizon SPE-DEX® 4790 Automated Extraction System.
- Attachment II: Horizon Extraction Method 46.2 and Purge Method 46.9.
- Attachment III: GC/MS Operating Conditions.
- Attachment IV: APEX ProSep™ Operating Conditions.

1.5 Interferences

1.5.1 Sources of possible contamination are glassware, equipment and reagents. Analysis of reagent blanks and matrix blanks can determine if contamination is present.

1.5.2 Contamination can occur on the Horizon extraction system if a sample containing high concentrations of analytes is immediately followed by extraction of a sample containing low levels of analytes. Care must be taken to replace ALL consumable parts, including the luer adapter for the speedisk and a thorough rinsing of the dry disk apparatus.

1.5.3 Carryover on the APEX from the syringe is possible. Suspected high level extracts should be injected last, and followed by hexane rinse injections. After every 5 sample extract injections, a hexane rinse should be injected. If a sample extract contains low level analyte concentrations after immediately following a high level extract, the extract should be reinjected after a hexane rinse injection to verify there is no carryover.

2. Safety and Environmental Considerations

2.1 Many chemicals used in the preparation of samples and standards are potentially hazardous. Consult the Material Safety Data Sheets (MSDS) for storage, handling and disposal advice.

2.1.1 Acetone
2.1.2 Ethyl Acetate
2.1.3 Methanol
2.1.4 Hexane

2.2 The toxicity and carcinogenicity of chemicals used in this method have not been fully established. Regard each chemical as a potential health hazard. Minimize personal exposure by wearing safety glasses, a lab coat and impervious gloves when conducting this analysis. Avoid the release of chemicals into the laboratory environment by working in a fume hood when procedures are likely to produce noxious fumes, and by disposing of waste properly.

3. Sample Handling

3.1 The samples must be refrigerated at 4°C from the time of collection until extraction, a period of not more than 5 business days.

4. Apparatus and Materials

4.1 Equipment and Supplies

4.1.1 Sample bottles: Amber glass, 1 liter I-Chem, disposable or washed and solvent rinsed, capped with Teflon™ or foil caps.

4.1.2 Horizon SPE-DEX® 4790 automated extractor and all associated glassware, manufactured by Horizon Technologies:
- Cap adapters, 33 x 430 for 1L ICHEM bottle and 53 x 400 for wide mouth bottles.
- VOA vial, 40 mL.
- 19/22 Adapter for 40ml VOA vial.
- Luer adapter for Speedisk

4.1.3 Aluminum foil.

4.1.4 Turbo evaporator: Turbovap® II (ASE). Zymark corporation.

4.1.5 Pipets: Pasteur, glass disposable, 9” length.

4.1.6 Dionex (ASE) 40 mL stemmed tubes, part number: 055442.

4.1.7 Horizon Dry Disk filter assembly with 65mm DryDisk Separation Membrane. Horizon Technologies.

4.1.8 Speedisk, H2O-Phillic DVB. J.T. Baker.

4.1.9 Hewlett Packard 6890 gas chromatograph with HP 5973 mass selective detector, HP enviroquant data handling system, APEX ProSep injector, HP 6890 Series autosampler. Analytical column: HP5MS capillary column 30 m x 0.25 mm x 0.25 μm.

4.1.10 APEX precolumn: Midi II with BPX5 fiber.

4.2 Reagents and Standards

4.2.1 Acetone, methanol, ethyl acetate, hexane: pesticide quality, distilled in glass or nanograde.

4.2.2 Reagent water: bottled, suitable for gas chromatography or deionized water filtered through 0.45 micron filter and carbon cartridge.

4.2.3 Matrix water: Evian® brand bottled water or ground water.

4.2.4 Stock standard: Prepare by weighing 10 to 25 milligrams into a 28 mL vial and pipette in a suitable quantity of ethyl acetate to obtain 1.00 mg mL^{-1}. Prepare simazine in 0.10 mg mL^{-1} concentration.

4.2.5 Primary Standard Dilution Standard (PDS): Prepared from individual stock standards. Add 250 µL of each stock standard to a 25 mL volumetric flask and bring to volume with hexane to obtain 10 ppm.

4.2.6 Internal Standard Fortification Solution (ISFS): Prepare from the purchased internal standard, a 5.0 ppm solution in hexane containing: Phenanthrene-d10, Chrysene-d12 and Acenapthene-d10. Other internal standards such as Perylene-d12 may be included in this solution, but not used for this method. This solution is used in the preparation of the calibrations standards, fortification of the water sample extracts, and for preparing the internal standard dilution solution.

4.2.7 Process control Spiking Solution: Prepare a 5.0 ppm metazachlor solution by adding 500 µL of the metazachlor stock standard to a 100.0 mL volumetric flask and bringing to final volume with acetone. This solution is added to every water sample prior to preparation and extraction of this method.

4.2.8 PDP Marker Compound Spiking Solution: Prepare a spiking solution so each analyte concentration is 2 LOQ by adding the appropriate volume of the Neat standard to a 25.0 mL volumetric flask and bringing to final volume with acetone. 100 µL of this solution is added to create the matrix spike.

Analyte	[Spike] ppb	Vol. of neat to add (µL)
Simazine	2000	50
Metribuzin	2000	50
Acetochlor	1000	25
Triallate	2000	50
Trifluralin	3000	75
Diazinon	2000	50
Tebupirimphos	2000	50
DCPA (Dacthal)	2000	50
Propiconazole	4000	100
Boscalid	6000	150
Tebuthiuron	2000	50
Cis-permethrin	3000	75
Dichlobenil	300	7.5
Clomazone	2000	50

4.2.9 Internal Standard Dilution Solution: Prepare a 0.50 ppm solution by adding 5 mL of the 5 ppm Internal Standard Fortification Solution to a 50 mL volumetric flask and bringing to volume with hexane. This solution is used to bring dilutions of extracts to volume in order to maintain a 0.5 ppm internal standard concentration.

4.2.10 Calibration Standards: Prepare calibration standards at concentrations of 10, 20, 50, 100 and 200 ppb as follows:

[Calibration Stnd]	Vol. PDS to add	Final Volume	Vol. ISFS to add
2000 ppb	1000 µL	5.0 mL	500 mL
1000 ppb	500 µL	5.0 mL	500 µL
500 ppb	250 µL	5.0 mL	500 µL
250 ppb	125 µL	5.0 mL	500 µL
200 ppb	100 µL	5.0 mL	500 µL
100 ppb	50 µL	5.0 mL	500 µL
50 ppb	25 µL	5.0 mL	500 µL
20 ppb	10 µL	5.0 mL	500 µL
10 ppb	5 µL	5.0 mL	500 µL

5. Sample Preparation

5.1 Warm the sample to room temperature. Measure 1000 mL of sample and transfer to amber 1L bottle.

5.2 Warm the PDP marker compound spiking solution to room temperature and vortex just prior to pipetting. Add 100 µL of the Marker Spiking Solution to 1 L of Evian bottled water or ground water for the matrix spike. **Do not allow PDP marker spiking solution to sit out for more than 1 hour.**

5.3 Warm the Process control Surrogate Spiking Solution to room temperature and vortex just prior to pipetting. Add 100 µL of the 5.0 ppm process control spiking solution to each sample, reagent blank, matrix blank and matrix spike. **Do not allow process control spiking solution to sit out for more than 1 hour.**

5.4 Shake all samples and QC to thoroughly mix.

6. Procedure

6.1 Loading sample onto extractor:

6.1.1 Place a 1 x 1 inch aluminum foil piece over the top of the bottle and screw on a clean 33 x 430 cap adapter.

6.1.2 Follow Attachment I on system checks before proceeding to loading the samples.

6.1.3 For Neutrals, load method 46.2 (Attachment II) into the controller for the extractors that are being used. See Attachment I for detailed instructions.

6.1.4 Attach a Hydrophillic speedisk to the extractor deck using a clean luer disk adapter.

6.1.5 Attach a clean VOA bottle and adapter to the collection site. Make sure the adapter sits tightly onto the VOA vial.

6.1.6 Load the sample onto the Horizon extractor following directions in Attachment I. **DO NOT turn the bottle counter-clockwise.**

6.1.7 Load the remaining samples in the same manner.

6.1.8 Once all the samples have been loaded, start the extraction by pressing the start button on each individual extractor. All the extractors need to be in the same prewet step for optimal vacuum on the waste collection bottle. If one sample finishes the extraction early, **DO NOT** load another sample on that unit until all the samples have finished.

6.1.9 Once the samples have all completed the extraction, remove the VOA collection vial and replace the adapter with a screw top cap.

6.2 Removal of water from sample extract:

6.2.1 Turn on vacuum and adjust so that the pressure gauge on vacuum manifold is in the green range, optimally at -15" Hg.

6.2.2 Vortex the sample in the VOA vial.

6.2.3 Place a new drydisk membrane into the filter holder.

6.2.4 Attach a clean adapter to a 40 mL Dionex tube and attach to filter holder.

6.2.5 Pour sample into filter holder, turn stop-cock to allow sample to flow through drydisk.

6.2.6 Rinse the VOA vial with a small volume of hexane and pour into filter holder. Repeat one more time.

6.2.7 Once extract has collected into dionex tube, fully open stopcock to allow for any residual hexane to flow through.

6.2.8 Remove Dionex tube and clean filter holder by pouring off remaining water, discarding the drydisk and rinsing the filter holder and reservoir walls with acetone. Dry with a clean Kimwipe.

Acceptable stopping point for overnight storage.

6.3 Concentration:

6.3.1 Check water bath level and set turbovap to 35°C and select sensor option for blowdown.

6.3.2 Set nitrogen regulator to a pressure of approximately 45 psi and pressure on the turbovap to approximately 10 psi.

6.3.3 Place Dionex tubes in evaporation cell and push start.

6.3.4 Turbovap will beep when sample concentrates to approximately 0.75 mL. Remove from evaporator. If not at appropriate level, press cell button to turn off flow and then press cell button to start over again.

6.3.5 When removing the Dionex tubes from the turbovap cells, be careful to not let water drip into the remaining tubes.

6.4 Addition of internal standard:

6.4.1 Add 100 μL of internal standard containing 5 ppm of phenanthrene-d10, chrysene-d12 and acenapthene-d10 to provide a final extract concentration of 0.5 ppm internal standard.

6.4.2 Bring to final volume of 1.0 mL with hexane.

6.4.3 Vortex the sample and then transfer an aliquot into a GC vial containing a microvial insert; cap with a Teflon faced silicone rubber autosampler crimp cap. Transfer the remaining sample to a 5 mL vial or another GC vial and store in the refrigerator. Sample is now ready for analysis by GC/MS.

6.5 GC/MS operation and calibration:

6.5.1 All PDP GC/MS samples are run on GMC.

6.5.2 Attachment III contains the GC/MS operating conditions. Attachment IV contains the APEX ProSep injector operating conditions. Table 2 contains the retention times, identifying masses for each analyte and related internal standard analyte for quantitation.

6.5.3 The GC/MS needs to be tuned each day of operation. Use PFTBA, following tuning criteria to show that the instrument is functioning properly.

6.5.4 The instrument must be recalibrated with each mass spec. run using concentrations of 10, 20, 50, 100, 200, 250, 500, 1000 and 2000 ppb. Each standard contains the internal standard, phenanthrene d-10 and Chrysene-d12 at 500 ppb.

6.5.5 A 500 ppb PDP standard must be run at the end of each extraction batch of samples to check the quantitation of compounds. If any compounds are detected, the quantitation of the 50 ppb standard must be ± 20% of the true value or else a new calibration curve must be performed

6.5.6 For the linear curve to be valid, the r^2 value of the curve must be greater than 0.990.

6.5.7 Sample concentrations must be within 20% of the highest level of the concentration range of the curve, or else dilutions must be made. Dilutions must be made using the Internal Standard Dilution Solution to maintain the internal standard concentration at 500 ppb.

6.6 GC / MS confirmation requirements:

6.6.1 The relative ion intensities may change because of performance or operating conditions, so the 500 or 1000 ppb calibration standard should be used to update retention times and intensities.

6.6.2 Retention times of the analyte of interest in the standard and the retention time of the same analyte in the sample must be within ± 0.10 minutes.

6.6.3 Structurally significant ions chosen for quantitation and identification must have intensities 3 times baseline.

6.6.4 Relative abundances of structurally significant ions used for confirmation should agree to ± 20% (absolute) of the standard injected that day. For ions where the target ratios are < 20%, the relative percent must be used.

6.6.5 For compounds that do not meet the above criteria because of the nature of the compound, co-elution problems or ability of the instrument, a comment must be included explaining the exception and included in the hardcopy of the batch.

6.7 Calculations:

6.7.1 Percent Recovery matrix spike samples

$$\%\,\text{Recovery} = \left(\frac{\text{SpSa - Sa}}{\text{Sp}}\right)100$$

where: SpSa : Spiked sample concentration
Sa : Sample concentration
Sp : Spike concentration added to sample.

6.7.2 Determine the concentration of the individual compounds in the sample by:

ppb = $[(A_x)(Q_{is}) / (A_{is})(RF)(V)]$

AX = integrated abundance of the quantitation ion of the analyte in the extract..
Qis = total quantity (in µg) of internal standard added to the water extract.
Ais = integrated abundance of the quantitaion ion of the internal standard in the extract.
RF = mean response factor of analyte from the calibration. RF is a unitless value.
V = original sample volume in mLs

6.7.3 Report results in parts-per-billion (ppt, ng/L) without correction for recovery data.

7. Critical Control Points

7.1 A calibration curve is generated from the nine standard levels (see Section 6.5.4). Not all of the analytes will be detected at every level. All compounds need to be detected at their LOD levels. A minimum of 3 points is needed for quantitation using a linear equation. Some analytes will overwhelm the system at the 2000 ppb level, delete that level and use the highest concentration level actually used in the calibration to determine the new quantitation range if dilutions are needed.

7.2 Spiked samples: These samples will be entered in the Laboratory Information Management System and plotted in Northwest Quality Analyst. After 20 points, limits will be calculated. Samples that fall outside the limits will require corrective action.

7.3 Steps in the method and attachments in bold and underlined are considered critical control points and must be adhered to.

7.4 The Horizon extraction system does not work well below 60º F, the Teflon valves will begin to leak. The sample will slowly drip into the prewet steps, resulting in loss of recovery. **DO NOT** run extractors when laboratory air temperatures are below 60˚ F.

7.5 Avoid **ANY** injection of methanol into the APEX system, the methanol will destroy the BPX liner and that may in turn destroy the analytical column and lead to a very dirty source.

8. System Maintenance

8.1 All maintenance will be recorded in the APEX GC/MS log book located on the shelf above the computer in the instrument room.

8.2 Injector septum must be replaced after every instrument batch run.

8.3 The APEX precolumn must be replaced as needed, or every month if used heavily. Some indications of needed maintenance are:
- 8.3.1 Calibration curve loses linearity of analytes that are typically linear.
- 8.3.2 Calibration curve does not last for 24 hours.
- 8.3.3 Increased baseline noise.
- 8.3.4 Appearance of extraneous peaks in the hexane rinses.
- 8.3.5 Increased peak tailing, splitting of peaks or decrease in response.

8.4 Trim injector end of analytical column as needed. The APEX GC/MS does not have a guard column, so the injector end of the analytical column may need to trimmed after a large number of dirty extract injections. Some indications of needed trimming are:
- 8.4.1 Replacing septum and precolumn does not solve the problem.
- 8.4.2 Inadequate sensitivity.
- 8.4.3 Inadequate peak resolution.

8.5 The source occasionally needs to be cleaned. The large volume injection can dirty the source more rapidly than smaller injections. Some indications of the source needing to be cleaned:
- 8.5.1 Replacing septum, precolumn and trimming analytical column does not solve the problem.
- 8.5.2 Failure to tune or difficulty in isotope masses being detected.

8.6 Replace analytical column. Some indications that the analytical column may need to be replaced:
- 8.6.1 Replacing septum, precolumn, trimming analytical column or cleaning the source does not solve the problem.

8.6.2 Power failure when the analytical column and precolumn were at maximum temperature. When the power goes off, the electronic pressure control shuts off and helium flow ceases, destroying the column. Trimming the injector end often corrects this, but after numerous trimmings, the column may need to be replaced.

8.7 Syringe and turret errors:

8.7.1 The large volume of sample being injected often plugs, or makes the syringe "sticky", especially following the heavy sediment groundwater samples. Prior to the start of every batch, rinse the syringe with several methanol rinses. **DO NOT** pull the plunger completely out. After the methanol rinses, rinse the syringe with the 30/70 acetone / iso-octane rinse vial solution. **<u>Any residual methanol will destroy the BPX lined precolumn.</u>**

8.7.2 Power failures will frequently result in turret error messages. To correct, exit all of the Chemstation software, turn the autosampler control box off, wait about 20 seconds, turn the control box back on and then reenter Chemstation. Reload method and sequence and start injecting.

Table 1: Level of Detection (LOD) and Level of Quantitation (LOQ) in ppt (ng L^{-1})

Compound	LOD (ppt)	LOQ (ppt)
Acetochlor	10	50
Alachlor	10	50
Atrazine	10	50
Boscalid	100	30
Chlorothalonil	30	100
Chlorpyrifos	30	100
Cis-Permethrin	50	150
Clomazone	30	100
Cyanazine	50	200
DCPA (Dacthal)	30	100
Deethylatrazine	10	50
Deisopropylatrazine	50	200
Diazinon	30	100
Dichlobenil	5	15
Dimethenamid	10	50
Dimethoate	50	200
EPTC	30	100
Ethafluralin	30	150
Fluometuron	50	150
Fonofos	30	150
Malathion	30	100
Methyl Parathion	30	100
Methidathion	100	300
Metolachlor	15	70
Metribuzin	30	100
Myclobutanil	50	200
Pendimethalin	30	100
Phorate	30	100
Phorate oxon	50	150
Phorate sulfone	100	300
Phorate sulfoxide	100	300
Prometon	30	100
Propachlor	30	100
Propanil	30	100
Propazine	30	100
Propiconazole	50	200
Simazine	30	100
Tebuconazole	50	200
Tebupirimphos	30	100
Tebuthiuron	30	100
Terbufos	30	150
Tetraconazole	30	150
Trans-Permethrin	50	150
Triallate	30	100
Trifluralin	30	150
Triticonazole	500	1000

Table 2: Retention Times and Mass Analysis

Analyte	RT (min)	Masses	Internal Standard
Acetochlor	15.46	146, 162, 132, 223	Phenanthrene-d10
Alachlor	15.82	160, 188, 146, 238	Phenanthrene-d10
Atrazine	13.06	200, 215, 202, 173	Phenanthrene-d10
Boscalid	33.26	140, 112, 142, 342	Chrysene-d12
Chlorpyrifos	17.67	199, 197, 314, 258	Phenanthrene-d10
Chlorothalonil	14.30	266, 264, 268, 231	Phenanthrene-d10
Cis-Permethrin	31.70	183, 163, 165, 127	Chrysene-d12
Clomazone	13.12	125, 204, 127, 205	Phenanthrene-d10
Cyanazine	17.81	225, 173, 240, 212	Phenanthrene-d10
DCPA (Dacthal)	17.91	301, 299, 332, 221	Phenanthrene-d10
Deethylatrazine	11.83	172, 174, 187, 145	Phenanthrene-d10
Deisopropylatrazine	11.72	173, 158, 145, 110	Phenanthrene-d10
Diazinon	13.88	137, 179, 152, 304	Phenanthrene-d10
Dichlobenil	9.28	171, 173, 100, 136	Acenapthene-d10
Dimethoate	12.76	87, 125, 93, 229	Phenanthrene-d10
EPTC	9.31	128, 132, 86, 189	Phenanthrene-d10
Ethafluralin	11.73	276, 316, 292, 333	Phenanthrene-d10
Fluometuron	11.39	72, 232, 187, 145	Phenanthrene-d10
Fonofos	13.62	109, 137, 246, 110	Phenanthrene-d10
Malathion	17.22	125, 93, 173, 127	Phenanthrene-d10
Malathion oxon	15.44	127, 109, 142, 268	Phenanthrene-d10
Metazachlor	19.37	132, 133, 209, 117	Phenanthrene-d10
Methyl Parathion	15.54	109, 125, 263, 233	Phenanthrene-d10
Methidathion	20.82	145, 85, 93, 125	Phenanthrene-d10
Metolachlor	17.42	162, 238, 240, 211	Phenanthrene-d10
Metribuzin	15.30	198, 144, 199, 103	Phenanthrene-d10
Myclobutanil	24.71	179, 150, 206, 288	Chrysene-d12
Pendimethalin	19.52	252, 119, 161, 281	Phenanthrene-d10
Phorate	12.23	75, 121, 260, 231	Phenanthrene-d10
Phorate oxon	11.22	171, 75, 111, 138	Acenaphthene-d10
Phorate sulfone	17.18	97, 125, 153, 199	Phenanthrene-d10
Phorate sulfoxide	16.82	97, 125, 153, 199	Phenanthrene-d10
Prometon	12.85	168, 210, 225, 183	Phenanthrene-d10
Propachlor	11.28	120, 77, 176, 211	Phenanthrene-d10
Propanil	15.21	161, 163, 57, 217	Phenanthrene-d10
Propazine	13.17	214, 172, 229, 187	Phenanthrene-d10
Propiconazole	27.89	173, 175, 259, 261	Chrysene-d12
Simazine	12.92	201, 186, 173, 138	Phenanthrene-d10
Tebuconazole	28.21	125, 250, 127, 163	Chrysene-d12
Tebuprimiphos	14.56	152, 137, 234, 318	Phenanthrene-d10
Tebuthiuron	10.41	156, 171, 74, 88	Acenaphthene-d10
Terbufos	13.50	231, 153, 186, 288	Phenanthrene-d10
Tetraconazole	18.22	336, 338, 159, 171	Phenanthrene-d10
Trans-Permethrin	31.88	183, 163, 165, 127	Chrysene-d12
Triallate	14.33	86, 268, 270, 128	Phenanthrene-d10
Trifluralin	11.91	306, 264, 248, 335	Phenanthrene-d10
Triticonazole	30.10	235, 115, 217, 219	Chrysene-d12

Attachment I: Operation of the Horizon SPE-DEX® 4790 Automated Extraction System.

System Startup Check Procedure

1. Empty waste water bottle and solvent waste bottle.

2. Fill solvent bottles if needed and make sure solvent lines are not kinked and line connections are secure.

3. Turn on vacuum pump.

4. Turn on nitrogen gas supply, line regulator should be set for 80 psi. On the Horizon regulator bracket assembly, extractor (left) regulator should be set for 40 psi and the solvent bottle (right) regulator pressure needs to be set for 15 psi. (to adjust turn the right knob until the appropriate pressure is obtained).

5. Using the check valve tool, make sure the check valve is free and not sticking.

6. Before proceeding, make sure the vacuum pump pressure is –25" Hg and the gauge pressure on the waste solvent recovery bottle is –15"Hg.

7. Check that the sample flow valve is working. Load method **15** into the controller. To load a method:
   - 7.1 Press "select" button.
   - 7.2 Screen prompts "Extractor number". Input the extractor number by typing "**1..6**" (".." means thru; "." means and).
   - 7.3 Press "Enter" button.
   - 7.4 Screen prompts "Method Number". Input valve check method by typing "**15**".
   - 7.5 Press "Enter" button.
   - 7.6 Screen reads: "Method loaded into extractor(s)" then "SPE-DEX / Controller / 4700 extractors".

8. Using a mirror to look into the extractor chamber, press the purge button on the extractor. You will hear a click and see the valve open. Repeat for each extractor. **BE SURE TO WEAR EYE PROTECTION** for this step. If the method is misloaded, pressing the purge button will shoot solvent upward!

9. **GENTLY** lower the disk holder platform and center the empty metal disk holder with metal screen using a luer adapter into the vacuum port. Very gently press down to ensure a tight seal. Take care to avoid touching the thermocouples on the back side. Gently raise platform into position. Attach an Erlenmeyer flask as the collection vessel. Loosely place a 50 mL VOA vial over the solvent spray outlet on top of the extractor.

10. On the controller, select all the controllers to run the purge method for Neutrals: **46.9**.

11. Press purge button on each extractor unit.

12. Discard the solvent collected in the Erlenmeyer flask, reconnect flask. Adjust solvent bottle pressure to –15”Hg if necessary and rerun purge method.

13. After second purge is run, verify that each extractor has collected the same volume of extract.

<u>Loading a Sample Bottle</u>

1. Load Neutrals extraction method **46.2** into the controller for every extractor that is going to be used for that method.

2. Place a 1” x 1” piece of aluminum foil over the mouth opening of the bottle. Screw on a 33 x 430 cap adapter over the foil. When the adapter is in place, the aluminum foil should be taut.

3. Invert the sealed bottle. Make sure no bubbles are rising around the neck of the adapter indicating a poor seal.

4. Guide the bottle so that the solvent rinse stem will be directly under the bottle adapter. The bottle should be perpendicular to the bench.

5. GENTLY lower and then firmly push the sample into the bottle holder. The black o-ring inside of the bottle holder ensures a leak tight seal is made between the bottle cap adapter and the bottle holder.

6. Turn the bottle CLOCKWISE 3/4 of the way around to break an opening in the foil and allow the sample to flow. The trapped air bubbles should release. If there are no bubbles, give the bottle a gentle shake and give the bottle another 1/4 turn clockwise.

   Note: <u>NEVER</u> turn the bottle counter clockwise – it will cause the cap to loosen resulting in a leak and loss of sample.

7. Repeat loading for all the samples and extractors to be used.

8. Once all the samples have been loaded, press the START button on each extractor. All the extractors need to be at the same time during the prewet steps to optimize the vacuum.

9. Remove the bottle by gently pulling it straight up out of the bottle holder. **DO NOT** leave the bottles in the bottle holder.
10. Load the next samples after **all** of the samples have finished.

<u>Shut Down Procedure</u>

1. Attach the metal disk holder with metal screen to the vacuum port. DO NOT use the luer adapters. Attach an Erlynmeher flask as the collection vessel.

2. Pour hot water into the empty disk holder and fill half way. While holding down the ABORT key, press the PURGE key. Release once the light goes on and the water begins to flow into the Erlenmeyer flask. If you hear a click and the water does not flow into the flask, press the ABORT key and try again. This step will flush and clean the elute check valve from any solvent or debris.

3. Turn off the vacuum pump and vent it by disconnecting the line on the pump to the waste bottle.

4. Turn off the gas supply and open the solvent bottle (right) regulator on the regulator bracket assembly.

5. Turn off the power supply on the controller.

Attachment II: Horizon Extraction Method 46.2 and Purge Method 46.9

Extraction Method 46.2:

Method 46.2

Step	Solvent	Soak Time	Dry Time
Prewet			
Prewet 1	Acetone	1:30	1:30
Prewet 2	EtOAc	1:30	1:30
Prewet 3	Hexane	1:30	1:30
Prewet 4	MeOH	1:30	0:00
Prewet 5	H2O	1:30	0:00
No Wash Step			
Process Sample			
Air Dry			0:05
Rinse			
Rinse 1	Acetone	3:00	0:10
Rinse 2	EtOAc	1:30	0:10
Rinse 3	Hexane	1:30	0:10
Rinse 4	Hexane	1:30	0:10
Rinse 5	Hexane	1:30	0:30

Purge Method 46.9:

Method 46.9

Step	Solvent	Soak Time	Dry Time
Prewet			
Prewet 1	Acetone	0:00	0:05
Prewet 2	EtOAc	0:00	0:05
Prewet 3	Hexane	0:00	0:05
Prewet 4	MeOH	0:00	0:05
Prewet 5	H2O	0:00	0:05
No Wash Step			
Process Sample			
Air Dry			0:05
Rinse			
Rinse 1	Hexane	0:00	0:05
Rinse 2	EtOAc	0:00	0:05
Rinse 3	Acetone	0:00	0:10

Attachment III: GC/MS Operating Conditions

Acquisition Parameters

1. Column: HP5MS 30 m x 0.25 µm x 0.25 mm ID+.

2. Carrier gas: helium at constant flow of 1.5 mL min^{-1}, 12.7 psi at 50 °C, average velocity = 45 cm sec^{-1}.

3. Equilibration time: 4.00 min.

4. Solvent delay: 8.50 min.

6. Scan range: 45-450 amu.

7. Valves: 7, switching.

8. Injection volume: 20 µL.

9. Solvent rinses:

   9.1 Sample pumps – 3

   9.2 Pre inj. solv A – 1

   9.3 Pre inj. solv B – 1

   9.4 Post inj. solv A – 1

   9.5 Post inj. solv B – 1

   9.6 viscosity delay - 5 sec

   9.7 Plunger speed - slow

Zone Temperatures

1. Injection temperature - 250˚C.

2. Interface temperature - 280˚C.

3. MS quad temperature - 150˚C.

4. MS source temperature - 230˚C.

5. Initial oven temperature - 50˚C.

6. Initial time - 4.00 min.

7. Oven ramp 1 - 25˚C $min.^{-1}$

8. Final temperature 1 - 180˚C.

9. Final time 1 - 4.00 min.

10. Oven ramp 2 - 5˚C $min.^{-1}$

11. Final temperature 2 - 190˚C.

12. Final time 2 - 8.00 min.

13. Oven ramp 3 - 10˚C $min.^{-1}$

14. Final temperature 3 - 270˚C.

15. Final time 3 - 9.00 min.

Attachment IV: APEX ProSep Operating Conditions

Precolumn Mode Program

1. GC Split - 0.00 min.
2. Splitless - 0.30 min.
3. GC Split - 7.00 min.
4. ProSep Split - 36.00 min.

Precolumn Temperature Program

1. Initial temperature - 60˚C.
2. Initial time - 0.10 min.
3. Precolumn ramp 1 - 150˚C min.$^{-1}$
4. Final temperature 1 - 280˚C.
5. Final time 1 - 35.26 min.
6. Precolumn ramp 2 - 150˚C min.$^{-1}$
7. Final temperature 2 - 290˚C.
8. Final time 2 - 3.26 min.